

INTERNATIONAL JOURNAL OF PROBIOTICS & PREBIOTICS
VOLUME 3 NUMBER 1

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International Journal of Probiotics & Prebiotics 3(1): 1-6

1-6 Effects Of Feeding Spray dried Metabolite Of *Lactococcus Lactis* Subsp. *Lactis* – RW18 In Post weaning Rats

T.C. LOH, H. A. HARUN, H. L. FOO AND F. L. LAW

ABSTRACT: None information was available on the effects of using spray-dried metabolites of *Lactococcus* spp. isolated from our local fermented food, 'tapai ubi'. Thus, this study was aimed to investigate the effects of feeding spray-dried metabolite (SDM) produced by *L. lactis* subsp. *lactis*- RW18 on faecal pH, Enterobacteriaceae bacterial and LAB counts in faeces, plasma cholesterol concentration and growth performance of post weaning rats treated with different levels of SDM as additive in basal diet. A total of twenty-four 4 weeks old Sprague Dawley (male) rats were housed individually in each cage for each treatment. The rats were weighed weekly for four weeks. Water and feed were supplied ad libitum and feed intake was measured daily and provided in mash form. The animals were randomly assigned to three groups: (i) basal diet (as control); (ii) 0.25% (w/ w) SDM; (iii) 0.50% (w/w) SDM. All groups were acclimatized to the respective diets for one week before the experiment started. In terms of growth performance, faecal lactic acid bacteria (LAB) and Enterobacteriaceae counts, faecal pH and plasma cholesterol concentration, 0.25% SDM group had better performance than 0.50% SDM and control groups.

International Journal of Probiotics & Prebiotics 3(1): 7-14

7-14 Effect Of Probiotics *Lactobacillus Rhamnosus* Gg And New Isolate *Enterococcus Faecium* Ef2019 (Ccm 7420) On Growth, Blood Parameters, Microbiota And Coccidia Oocysts Excretion In Rabbits

MONIKA SIMONOVÁ, MIROSLAVA MARCINÁKOVÁ, VIOLA STROMPFOVÁ, KLAUDIA COBANOVÁ, SONA GANCARCÍKOVÁ, ZUZANA VASILKOVÁ AND ANDREA LAUKOVÁ

ABSTRACT: The effect of a commercial strain (*Lactobacillus rhamnosus* GG) and a new bacteriocinogenic, probiotic strain of rabbit origin, *Enterococcus faecium* CCM 7420 (our isolate) was tested on growth performance, blood parameters, faecal microflora, organic acids concentrations and *Eimeria* sp. oocysts occurrence in rabbits model experiment. Average daily gain was increased in both experimental groups by 21.9 % and 12.5 %. Significant reduction of bacteria was recorded due to both probiotics; *E. coli*, (even haemolytic), coagulase-positive staphylococci, *Staphylococcus aureus*, pseudomonads and Clostridium-like bacteria were reduced. Administration of probiotics increased the faecal concentration of lactic, butyric and succinic acids and also of serum total protein and lipids in both experimental groups. *E. faecium* CCM 7420 reduced the occurrence of *Eimeria* sp. oocysts in faeces. Moreover, the higher counts of CCM 7420 strain (6.0 - 4.0 log₁₀ cfu/g) were recorded in the faeces during the whole experiment comparing to the counts of *L. rhamnosus* GG (3.4 – 1.1 log₁₀ cfu/g). Because lactobacilli occurred rarely in rabbits intestine, enterococci have better chance to be used as feed additive and/or probiotics in rabbit breeding.

International Journal of Probiotics & Prebiotics 3(1): 15-20

15-20 Antibiotic Susceptibility And *In Vivo* Studies Of Selected Probiotic Strains Of *Lactobacillus Casei*

VIJENDRA MISHRA, PAWAS GOSWAMI, RAJESH DABUR AND D. N. PRASAD

ABSTRACT: Probiotics are the dietary adjuncts providing health benefits to consumers. The selection of probiotics before incorporation in diet requires close scrutiny by *in vitro* as well as *in vivo* tests along with antibiotic susceptibility profiles. The present study was undertaken to check the susceptibility of three selected strains of *Lactobacillus casei* to commonly used antibiotics and evaluate the effect on fecal flora and beta-glucuronidase activity in experimental animals. All strains were resistant to amoxicillin, vancomycin, kanamycin, metronidazole, polymyxin B and furazolidone while sensitive to ampicillin, chloramphenicol, clindamycin, rifampicin and trimethoprim. The plasmid analysis showed lack of plasmid in all three strains. Feeding albino female mice revealed that *Lactobacillus* numbers increased initially for 10 days and remained static till 15th day. When feeding was stopped, strain NCDC17 was the most persistent but other groups recorded a drop in count, highest for strain Y (2 log cycles). During feeding though all groups showed a decrease in faecal b-glucuronidase activity, NCDC 17 showed most stable reducing ability.

International Journal of Probiotics & Prebiotics 3(1): 21-24

21-24 Toxicity Of A Ferment Used In The Production Of Probiotic Capsules

THOMAS PARADATHATHU, K. PHILIP AND B. Y. HASHIM

ABSTRACT: : Male Sprague Dawley rats were fed orally for 7 days with a live lactic acid bacteria containing fermented paste used for preparing probiotic capsules at concentrations of 2.4×10^8 CFU/ml, 1.8×10^8 CFU/ml and 1.2×10^8 CFU/ml. During this period, rat blood was analyzed for levels of urea and the enzyme alanine aminotransferase (ALT) activity, as surrogate markers for kidney and liver toxicity respectively. The gross morphology of the animals was also observed. At the end of 7 days, the animals were sacrificed and the heart, liver and kidneys were removed and the organ weight to body-weight ratios were calculated. There was no significant difference in physical appearance, blood urea levels and blood ALT activity between the control group fed with water and the 3 groups of animals that were fed different dilutions of the paste. There were also no significant differences between the ratio of kidney, heart and liver weight to body-weight of the animals between the different groups. The results indicate that a paste containing a high concentration of lactic acid bacteria did not produce toxicity in rats as measured by levels of blood urea, ALT activity and ratios of organ weight to body weight. Therefore, it can be concluded that probiotic products containing live lactic acid bacteria can generally be considered as safe.

International Journal of Probiotics & Prebiotics 3(1): 25-30

25-30 Toxicity Of A Ferment Used In The Production Of Probiotic Capsules

MARISA CASTROA, MATÍAS MOLINAA, MÓNICA SPAROB, MARCELA MANGHI

ABSTRACT: *Enterococcus faecalis* CECT7121 is a non-pathogenic strain that, after the intragastric (ig) administration, it colonizes the intestinal tract of BALB/c mice and modulates the innate systemic immune response by inducing the synthesis of homeostatic cytokines (IL-12 and IL-10). In this work we analyzed the adjuvant properties of *E. faecalis* CECT7121, as well as its influence on the Th1/ Th2 polarization during the specific immune response generated by the immunization with the vaccine of pediatric use DTPw (diphtheria, tetanus and whole-cell *Bordetella pertussis*). The study was carried out comparatively in naïve BALB/c mice and mice pre-treated ig with *E. faecalis* CECT7121 before the sc immunization with DTPw. The following parameters were assessed: the levels of antitetanus and

antidiphtheria antibodies, the proliferation of splenocytes and the levels of IFN γ , IL-5, IL-6 and IL-12 secreted in culture supernatants after the specific stimulation with tetanus and diphtheria toxoids. Those animals pre-treated with *E. faecalis* CECT7121 and immunized with DTPw displayed higher levels of secreted IFN γ , IL-6 and specific proliferation than untreated animals ($p < 0.05$). The levels of IL-5 and IL-12 as well as the IgG1/IgG2a ratios remained unaffected. Our results demonstrate that the administration of *E. faecalis* CECT7121 has an adjuvant effect and behaves as a pro-Th1 modulator inducing the synthesis of higher levels of IFN γ in the antitetanus and antidiphtheria responses at the systemic level.

International Journal of Probiotics & Prebiotics 3(1): 31-36

31-36 Analyses Of *Bifidobacterium*, *Lactobacillus*, And Total Bacterial Populations In Healthy Volunteers Consuming Calcium Gluconate By Denaturing Gradient Gel Electrophoresis And Real-Time PCR

KYLE ANDERSON, ZHONGTANG YU, JING CHEN, JULIE JENKINS, POLLY COURTNEY, AND MARK MORRISON

ABSTRACT: The objective of this study was to examine the impact of a common calcium gluconate supplement on the intestinal microbiota of healthy humans, with specific emphasis on bifidobacteria and lactobacilli. In this study, 12 healthy adults consumed 2.4 grams of calcium gluconate daily for three weeks. The fecal microbiota from weekly collected stool samples was subjected to three different analyses: community profiling by PCR-DGGE, generic profiling of *Bifidobacterium* and *Lactobacillus* by genus-specific PCR-DGGE, and quantification of these two genera by genus-specific real-time PCR. The gross PCR-DGGE profiles for each subject appeared to be unaffected by the gluconate consumption. Genus-specific PCR-DGGE profiles also showed unchanged bifidobacterial populations present in 9 of the 12 subjects throughout the study. Conversely, the lactobacilli-specific PCR-DGGE profiles did vary in most of the subjects during the study period. Real-time PCR assays showed an appreciable increase in bifidobacterial abundance (0.43 to 1.48 logs) while the gluconate supplement was consumed in 5 of the 12 subjects; however, this stimulatory effect diminished from 3 of the 5 subjects two weeks following the termination of gluconate supplementation. Lactobacilli exhibited similar trends, but to a lesser extent and for only three of the subjects. We conclude that the amount of gluconate (2.4 g/day) ingested for calcium supplementation purpose is probably not enough to confer any significant prebiotic effect for most healthy adults.

International Journal of Probiotics & Prebiotics 3(1): 37-46

37-46 Probiotics And *Helicobacter Pylori*: Preclinical And Clinical Studies

LIONETTI ELENA, MINIELLO VITO LEONARDO, FONTANA CLAUDIA, CUCCO CRISTINA, DE RUVO GIUSY, NICASTRO FRANCESCO, PAVONE PIERO, MAUROGIOVANNI GIOVANNI, AND FRANCAVILLA RUGGIERO

ABSTRACT: Current interest in probiotics as therapeutic agents against *Helicobacter pylori* is motivated not only by the clinical data showing efficacy of some probiotics in different gastrointestinal diseases but also by the increasing resistance of pathogenic bacteria to antibiotics and thus the increasing interest for alternative therapies by patients. The exact role of probiotics in this condition is still debated since only few randomized, double blind placebo-controlled trials are available and, of these few, some have recruited only a small number of patients. Moreover, these trials are difficult to compare for several reasons: differences in probiotic strains, dose, duration of treatment and formulation. Among probiotic applications, the reduction of antibiotic side effects is the best-documented indication as recently confirmed by meta-analyses, and now demonstrated even in children. At present, available data on

Helicobacter pylori indicates that probiotics are unable to eradicate the infection, but they could be useful in decreasing bacterial load and probably in improving dyspeptic symptoms. More studies on larger groups of patients are needed to clarify the exact role of probiotics in *Helicobacter pylori* infection.

International Journal of Probiotics & Prebiotics 3(1): 47-52

47-52 Role Of Microbiota In Oral Tolerance Induction And Infection

KAZUO TANAKA, SATOSHI NODA, HIROKI ISHIKAWA, AKIHIRO HATA, AND SADA AKI SAWAMURA

ABSTRACT: The roles of microbiota in oral tolerance induction and viral infection were investigated using germfree (GF) mice, which lacked the microbiota. In SPF mice, oral tolerance was induced both in Th1 (IgG2a production) and Th2 (IgG1 and IgE)-mediated immune responses. In contrast, in GF mice, oral tolerance was induced in the Th1 responses, but not in the Th2 responses. The GF mouse was thus considered to be a model of IgE-mediated allergic disorder. When GF were associated with *Bifidobacterium infantis*, oral tolerance was restored in the gnotobiotics to a level similar to that observed in SPF. In addition, when the SPF-BALB/c mice were infected with murine cytomegalovirus (MCMV), high frequencies of MCMV-specific CD8 memory T cells were detected in the lungs even at 8 months after infection. In contrast, the frequencies in the infected GF mice were significantly lower than those in SPF mice. The reconstitution of microbiota of MCMV-infected GF mice restored the frequencies of the memory T cells to the levels similar to those in SPF mice. These results may suggest probiotics may be a useful material not only for the treatment of IgE-mediated allergic disorder but also for maintaining the virus-specific memory T cells.

International Journal of Probiotics & Prebiotics 3(1): 53-60

53-60 Effect Of Four Probiotic Strains On Human Fecal Microbiota

MOHAMMAD ABDUL BAKIR, YUSUHIRO KOGA AND YOSHIMI BENNO

ABSTRACT: Six Japanese adults were given probiotics containing *Bacillus subtilis*, *Bifidobacterium bifidum*, *Enterococcus faecalis* and *Lactobacillus acidophilus*. Abundance of T-RFs (Terminal Restriction Fragments) related to probiotic bacterial species used in each subject and changes in the microbial intestinal community composition were monitored by T-RFLP (Terminal Restriction Fragment Length Polymorphism) fingerprints. All the probiotic species were able to survive in the intestine and tracked in the feces as indicated by the corresponding T-RFs. The relative area of observed T-RFs related to *B. subtilis*, *E. faecalis* and *L. acidophilus* were increased after the ingestion of probiotics including *Bifidobacterium* spp. T-RFs corresponding to the members of the genus *Clostridium*, *Ruminococcus*, *Eubacterium*, *Bacteroides* and *Streptococcus* were observed to be the dominant community in the fecal samples before as well as after receiving the probiotics. T-RFs related to the members of the genera *Clostridium*, *Ruminococcus* and *Streptococcus* increased after the ingestion of probiotics. Members of the genera *Bacteroides* and *Eubacterium* were observed to decrease with the ingestion of probiotic as revealed by the corresponding areas of the T-RFs of these genera. T-RFs related to uncultured bacteria represented 68 to 74% of the total fecal microbiota.

International Journal of Probiotics & Prebiotics 3(1): 61-64

61-64 Production Of Conjugated Linoleic Acid By Probiotic Microorganisms Utilizing Milk Fat

ARVIND, NIKHLESH KUMAR SINGH AND P.R. SINHA

ABSTRACT: High pressure liquid chromatography (HPLC) procedure was used to evaluate the production of conjugated linoleic acid (CLA) by probiotic microorganisms present in the dahi (D1) or individually by utilizing the milk fat and soybean oil as a source of linoleic acid (a substrate for CLA) and compared with normal dahi culture (mixed dahi culture, BD4) at different fat levels (3%, 4.5% and 6%). The probiotic microorganisms are able to produce more CLA as comparison to control dahi. Although the production of CLA at 6% level was highest among all fat levels, but it was not significantly differ except in probiotic dahi. The CLA production in soybean oil added skim milk was highest in culture, which was inoculated with probiotic microorganisms. In other parameters, with increase in fat level, titratable acidity increased while lactose content decreased in all groups. The production of more CLA may be due to the production of free fatty acids by lipolysis of milk fat and utilizing linoleic acid.